=> d 18 1-11 ti py au so

L8 ANSWER 1 OF 11 MEDLINE on STN
TI Detection of beta 2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET).
PY 2000
AU Angers S; Salahpour A; Joly E; Hilairet S; Chelsky D; Dennis M; Bouvier M
SO Proceedings of the National Academy of Sciences of the United States of America, **(2000 Mar 28)** 97 (7) 3684-9.
Journal code: 7505876. ISSN: 0027-8424.

L8 ANSWER 2 OF 11 MEDLINE on STN
TI Role of endocytosis in the activation of the extracellular signal-regulated kinase cascade by sequestering and nonsequestering G protein-coupled receptors.
PY 2000
AU Pierce K L; Maudsley S; Daaka Y; Luttrell L M; Lefkowitz R J
SO Proceedings of the National Academy of Sciences of the United States of America, **(2000 Feb 15)** 97 (4) 1489-94.
Journal code: 7505876. ISSN: 0027-8424.

L8 ANSWER 3 OF 11 MEDLINE on STN
TI The beta2-adrenergic receptor/**betaarrestin** complex recruits the clathrin adaptor AP-2 during endocytosis.
PY 1999
AU Laporte S A; Oakley R H; Zhang J; Holt J A; Ferguson S S; Caron M G; Barak L S
SO Proceedings of the National Academy of Sciences of the United States of America, **(1999 Mar 30)** 96 (7) 3712-7.
Journal code: 7505876. ISSN: 0027-8424.

L8 ANSWER 4 OF 11 MEDLINE on STN
TI **Agonist**-induced endocytosis and recycling of the gonadotropin-releasing hormone receptor: effect of beta-**arrestin** on **internalization** kinetics.
PY 1998
AU Vrecl M; Anderson L; Hanyaloglu A; McGregor A M; Groarke A D; Milligan G; Taylor P L; Eidne K A
SO Molecular endocrinology (Baltimore, Md.), **(1998 Dec)** 12 (12) 1818-29.
Journal code: 8801431. ISSN: 0888-8809.

L8 ANSWER 5 OF 11 MEDLINE on STN
TI Molecular mechanisms of G protein-coupled receptor desensitization and resensitization.
PY 1998
AU Ferguson S S; Zhang J; Barak L S; Caron M G
SO Life sciences, **(1998)** 62 (17-18) 1561-5. Ref: 22
Journal code: 0375521. ISSN: 0024-3205.

L8 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
TI **Arrestin** isoforms dictate differential kinetics of A2B adenosine receptor trafficking
PY **2000**
AU Mundell, Stuart J.; Matharu, Anne-Lise; Kelly, Eamonn; Benovic, Jeffrey L.
SO Biochemistry (**2000**), 39(42), 12828-12836
CODEN: BICHAW; ISSN: 0006-2960

L8 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
TI Selective regulation of endogenous G protein-coupled receptors by arrestins in HEK293 cells
PY **2000**
AU Mundell, Stuart J.; Benovic, Jeffrey L.

SO Journal of Biological Chemistry (**2000**), 275(17), 12900-12908
CODEN: JBCHA3; ISSN: 0021-9258

L8 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
TI Detection of β2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET)
PY **2000**
AU Angers, Stephane; Salahpour, Ali; Joly, Eric; Hilairet, Sandrine; Chelsky, Dan; Dennis, Michael; Bouvier, Michel
SO Proceedings of the National Academy of Sciences of the United States of America (**2000**), 97(7), 3684-3689
CODEN: PNASA6; ISSN: 0027-8424

L8 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
TI Role of endocytosis in the activation of the extracellular signal-regulated kinase cascade by sequestering and nonsequestering G protein-coupled receptors
PY **2000**
AU Pierce, Kristen L.; Maudsley, Stuart; Daaka, Yehia; Luttrell, Louis M.; Lefkowitz, Robert J.
SO Proceedings of the National Academy of Sciences of the United States of America (**2000**), 97(4), 1489-1494
CODEN: PNASA6; ISSN: 0027-8424

L8 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
TI **Agonist**-induced endocytosis and recycling of the gonadotropin-releasing hormone receptor: Effect of β- **arrestin** on **internalization** kinetics
PY **1998**
AU Vrecl, Milka; Anderson, Lorraine; Hanyaloglu, Aylin; McGregor, Alison M.; Groarke, Alex D.; Milligan, Graeme; Taylor, Philip L.; Eidne, Karin A.
SO Molecular Endocrinology (**1998**), 12(12), 1818-1829
CODEN: MOENEN; ISSN: 0888-8809

L8 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
TI Molecular mechanisms of G protein-coupled receptor desensitization and resensitization
PY **1998**
AU Ferguson, Stephen S. G.; Zhang, Jie; Barak, Larry S.; Caron, Marc G.
SO Life Sciences (**1998**), 62(17/18), 1561-1565
CODEN: LIFSAK; ISSN: 0024-3205

=> d his

(FILE 'HOME' ENTERED AT 16:44:05 ON 16 AUG 2004)

FILE 'MEDLINE, CAPLUS' ENTERED AT 16:44:24 ON 16 AUG 2004
L1 144 S ?ARRESTIN AND GREEN FLUORESCENT PROTEIN
L2 46 S L1 AND GPCR
L3 26 S L2 AND PY <2001
L4 524 S ?ARRESTIN AND INTERNALIZATION
L5 380 S L4 AND AGONIST
L6 102 S L5 AND GPCR
L7 41 S L6 AND PY <2001
L8 11 S L7 (L)L3

=>

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments
1	BRS	L1	0	gpcr adj arrestin	USPAT	2004/08/16 14:40	
2	BRS	L2	51860	gpcr pathway	USPAT	2004/08/16 14:40	
3	BRS	L3	38	l2 and arrestin	USPAT	2004/08/16 14:50	
4	BRS	L4	5	l3 and @py <2001	USPAT	2004/08/16 14:41	
5	BRS	L5	17890	l2 and detection	USPAT	2004/08/16 14:51	
6	BRS	L6	1223	l2 and gfp	USPAT	2004/08/16 14:51	
7	BRS	L7	14	l3 and gfp	USPAT	2004/08/16 14:51	

	U	1	PT	P	Document ID	Issue Date	Pages	Title
1	☐	☐	☐	☐	US 6159707 A	20001212	61	Sperm receptors
2	☒	☐	☐	☐	US 6110693 A	20000829	21	Methods of assaying receptor activity and constructs useful in such methods
3	☐	☐	☐	☐	US 5891646 A	19990406	23	Methods of assaying receptor activity and constructs useful in such methods
4	☐	☐	☐	☐	US 5591618 A	19970107	46	G protein-coupled receptor kinase GRK6
5	☐	☐	☐	☐	US 5532151 A	19960702	48	G protein-coupled receptor kinase GRK6

	Current OR	Current XRef	Retrieval Classif	Inventor	S	C	2	3	4	5
1	435/69.1	435/252.3; 435/254.11 ; 435/320.1; 435/325; 435/7.1; 530/350; 536/23.1; 536/23.5; 536/24.3; 536/24.31		Ronnett, Gabriele V. et al.	☒	☐	☐	☐	☐	☐
2	435/7.2	435/69.1; 435/7.1; 530/350; 536/23.4		Barak, Lawrence S. et al.	☐	☐	☐	☐	☐	☐
3	435/7.2	435/69.1; 435/7.1; 530/350; 536/23.4		Barak, Lawrence S. et al.	☒	☐	☐	☐	☐	☐
4	435/194	435/252.3; 435/320.1; 435/6; 536/22.1; 536/23.1; 536/23.2; 536/23.5		Chantry, David et al.	☒	☐	☐	☐	☐	☐
5	435/194	435/252.3; 435/320.1; 435/6; 536/22.1; 536/23.1; 536/23.2; 536/23.5		Chantry, David et al.	☒	☐	☐	☐	☐	☐

```
RE.CNT  5      THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
               ALL CITATIONS AVAILABLE IN THE RE FORMAT


=> d his

     (FILE 'HOME' ENTERED AT 14:31:58 ON 16 AUG 2004)

     FILE 'MEDLINE, USPATFULL, CAPLUS' ENTERED AT 14:32:25 ON 16 AUG 2004
L1             0 S DETECT? GPCR AND CONJUGATED ARRESTIN
L2           467 S GPCR AND ARRESTIN
L3            85 S L2 AND GFP
L4            56 S L3 AND PATHWAY
L5             4 S L4 AND PY <2001

=>
```

L5 ANSWER 1 OF 4 USPATFULL on STN
AN 2004:103678 USPATFULL
TI System for cell-based screening
IN Dunlay, R. Terry, New Kensington, PA, United States
Taylor, D. Lansing, Pittsburgh, PA, United States
Gough, Albert H., Glenshaw, PA, United States
Giuliano, Kenneth A., Pittsburgh, PA, United States
PA Cellomics, Inc., Pittsburgh, PA, United States (U.S. corporation)
PI US 6727071 B1 20040427
WO 9838490 19980903 <--
AI US 1999-380259 19991208 (9)
WO 1998-US3701 19980227
RLI Continuation-in-part of Ser. No. US 1997-810983, filed on 27 Feb 1997, now patented, Pat. No. US 5989835
PRAI US 1997-69329P 19971211 (60)
US 1997-69249P 19971211 (60)
DT Utility
FS GRANTED
LN.CNT 3071
INCL INCLM: 435/007.210
INCLS: 435/007.200; 435/004.000; 435/006.000; 435/007.100; 435/007.500; 435/288.400; 435/377.000; 435/375.000; 436/010.000; 436/017.000; 436/063.000; 436/164.000; 436/166.000; 436/172.000; 436/174.000; 436/517.000; 436/546.000; 382/255.000
NCL NCLM: 435/007.210
NCLS: 382/255.000; 435/004.000; 435/006.000; 435/007.100; 435/007.200; 435/007.500; 435/288.400; 435/375.000; 435/377.000; 436/010.000; 436/017.000; 436/063.000; 436/164.000; 436/166.000; 436/172.000; 436/174.000; 436/517.000; 436/546.000
IC [7]
ICM: G01N033-53
EXF 435/7.2; 435/7.21; 435/29; 435/40.5; 435/40.51; 435/288.3; 435/288.4; 435/4; 435/7.5; 435/6; 435/7.1; 435/183; 435/375; 435/975; 435/377; 436/546; 436/172; 436/800; 436/809; 436/10; 436/17; 436/164; 436/166; 436/174; 436/63; 436/517; 436/56; 356/300; 356/326; 356/328; 382/255; 382/141; 530/350
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 2 OF 4 USPATFULL on STN
AN 2000:113721 USPATFULL
TI Methods of assaying receptor activity and constructs useful in such methods
IN Barak, Lawrence S., Durham, NC, United States
Caron, Marc G., Hillsborough, NC, United States
Ferguson, Stephen S., London, Canada
Zhang, Jie, Durham, NC, United States
PA Duke University, Durham, NC, United States (U.S. corporation)
PI US 6110693 20000829 <--
AI US 1999-233530 19990120 (9)
RLI Continuation of Ser. No. US 1997-869568, filed on 5 Jun 1997, now patented, Pat. No. US 5891646
DT Utility
FS Granted
LN.CNT 1383
INCL INCLM: 435/007.200
INCLS: 536/023.400; 530/350.000; 435/007.100; 435/069.100
NCL NCLM: 435/007.200
NCLS: 435/007.100; 435/069.100; 530/350.000; 536/023.400
IC [7]
ICM: G01N033-52
ICS: C07H021-04; C12N015-12; C07K014-00
EXF 435/7.1; 435/176; 435/183; 435/6; 435/7.2; 435/69.1; 435/320.1; 536/23.4; 536/23.5; 530/350

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 3 OF 4 USPATFULL on STN
AN 1999:43394 USPATFULL
TI Methods of assaying receptor activity and constructs useful in such methods
IN Barak, Lawrence S., Durham, NC, United States
Caron, Marc G., Hillsborough, NC, United States
Ferguson, Stephen S., London, Canada
Zhang, Jie, Durham, NC, United States
PA Duke University, Durham, NC, United States (U.S. corporation)
PI US 5891646 19990406 <--
AI US 1997-869568 19970605 (8)
DT Utility
FS Granted
LN.CNT 1569
INCL INCLM: 435/007.200
INCLS: 536/023.400; 530/350.000; 435/079.100; 435/069.100
NCL NCLM: 435/007.200
NCLS: 435/007.100; 435/069.100; 530/350.000; 536/023.400
IC [6]
ICM: G01N033-52
ICS: C07H021-04; C12N015-12; C07K014-00
EXF 435/71; 435/174; 435/183; 435/6; 435/7.2; 435/69.1; 536/23.4; 536/23.5; 530/350
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:806798 CAPLUS
DN 130:63343
TI Assaying receptor activity using constructs expressing β-**arrestin** conjugates with green fluorescent protein
IN Barak, Lawrence S.; Caron, Marc G.; Ferguson, Stephen S.; Zhang, Jie
PA Duke University, USA
SO PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9855635	A1	19981210	WO 1998-US11628	19980604 <--
	WO 9855635	C2	20030508		
	W: AU, CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 5891646	A	19990406	US 1997-869568	19970605 <--
	CA 2305810	AA	19981210	CA 1998-2305810	19980604 <--
	AU 9877255	A1	19981221	AU 1998-77255	19980604 <--
	AU 759347	B2	20030410		
	EP 1015608	A1	20000705	EP 1998-925260	19980604 <--
	EP 1015608	B1	20040317		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	AT 262040	E	20040415	AT 1998-925260	19980604
	EP 1441032	A1	20040728	EP 2004-6057	19980604
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY				
	US 2004101887	A1	20040527	US 2003-628909	20030729
PRAI	US 1997-869568	A	19970605		
	EP 1998-925260	A3	19980604		
	WO 1998-US11628	W	19980604		
	US 1999-233530	A1	19990120		
	US 2000-631468	B1	20000803		

(FILE 'HOME' ENTERED AT 16:02:42 ON 16 AUG 2004)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 16:03:14 ON 16 AUG 2004

```
L1          458 S ?ARRESTIN AND GPCR
L2           34 S L1 AND GFP
L3            7 S L2 AND PY <2000
L4            0 S ARRESATIN AND GFP
L5          234 S ?ARRESTIN AND GREEN FLUORESCENT PROTEIN
L6           51 S L5 AND PY <2000
L7           40 S L6 AND G PROTEIN?
L8           34 S L6 AND G PROTEIN-COUPLED RECEPTOR
L9            0 S L8 AND DETECTION
L10           4 S L8 AND DETECT?
L11          12 S L8 AND ACTIVATION
```

=> d l11 1-12 ti py au so abs

L11 ANSWER 1 OF 12 MEDLINE on STN

TI Cellular trafficking of **G protein-coupled receptor**/beta-**arrestin** endocytic complexes.

PY 1999

AU Zhang J; Barak L S; Anborgh P H; Laporte S A; Caron M G; Ferguson S S

SO Journal of biological chemistry, **(1999 Apr 16)** 274 (16) 10999-1006.
Journal code: 2985121R. ISSN: 0021-9258.

AB beta-Arrestins are multifunctional proteins identified on the basis of their ability to bind and uncouple **G protein-coupled receptors** (GPCR) from heterotrimeric G proteins. In addition, beta-arrestins play a central role in mediating GPCR endocytosis, a key regulatory step in receptor resensitization. In this study, we visualize the intracellular trafficking of beta-arrestin2 in response to **activation** of several distinct GPCRs including the beta2-adrenergic receptor (beta2AR), angiotensin II type 1A receptor (AT1AR), dopamine D1A receptor (D1AR), endothelin type A receptor (ETAR), and neurotensin receptor (NTR). Our results reveal that in response to beta2AR **activation**, beta-arrestin2 translocation to the plasma membrane shares the same pharmacological profile as described for receptor **activation** and sequestration, consistent with a role for beta-**arrestin** as the agonist-driven switch initiating receptor endocytosis. Whereas redistributed beta-arrestins are confined to the periphery of cells and do not traffic along with activated beta2AR, D1AR, and ETAR in endocytic vesicles, **activation** of AT1AR and NTR triggers a clear time-dependent redistribution of beta-arrestins to intracellular vesicular compartments where they colocalize with internalized receptors. **Activation** of a chimeric AT1AR with the beta2AR carboxyl-terminal tail results in a beta-**arrestin** membrane localization pattern similar to that observed in response to beta2AR **activation**. In contrast, the corresponding chimeric beta2AR with the AT1AR carboxyl-terminal tail gains the ability to translocate beta-**arrestin** to intracellular vesicles. These results demonstrate that the cellular trafficking of beta-**arrestin** proteins is differentially regulated by the **activation** of distinct GPCRs. Furthermore, they suggest that the carboxyl-tail of the receptors might be involved in determining the stability of receptor/**betaarrestin** complexes and cellular distribution of beta-arrestins.

L11 ANSWER 2 OF 12 MEDLINE on STN

TI Real-time visualization of the cellular redistribution of **G protein-coupled receptor** kinase 2 and beta-**arrestin** 2 during homologous desensitization of the substance P receptor.

PY 1999

AU Barak L S; Warabi K; Feng X; Caron M G; Kwatra M M
SO Journal of biological chemistry, **(1999 Mar 12)** 274 (11) 7565-9.
Journal code: 2985121R. ISSN: 0021-9258.
AB The substance P receptor (SPR) is a **G protein-coupled receptor** (GPCR) that plays a key role in pain regulation. The SPR desensitizes in the continued presence of agonist, presumably via mechanisms that implicate **G protein-coupled receptor** kinases (GRKs) and beta-arrestins. The temporal relationship of these proposed biochemical events has never been established for any GPCR other than rhodopsin beyond the resolution provided by biochemical assays. We investigate the real-time **activation** and desensitization of the human SPR in live HEK293 cells using **green fluorescent protein** conjugates of protein kinase C, GRK2, and beta-**arrestin** 2. The translocation of protein kinase C betaII-**green fluorescent protein** to and from the plasma membrane in response to substance P indicates that the human SPR becomes activated within seconds of agonist exposure, and the response desensitizes within 30 s. This desensitization process coincides with a redistribution of GRK2 from the cytosol to the plasma membrane, followed by a robust redistribution of beta-**arrestin** 2 and a profound change in cell morphology that occurs after 1 min of SPR stimulation. These data establish a role for GRKs and beta-arrestins in homologous desensitization of the SPR and provide the first visual and temporal resolution of the sequence of events underlying homologous desensitization of a GPCR in living cells.

L11 ANSWER 3 OF 12 MEDLINE on STN
TI Using **green fluorescent protein** to understand the mechanisms of **G-protein-coupled receptor** regulation.
PY 1998
AU Ferguson S S
SO Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.], **(1998 Nov)** 31 (11) 1471-7. Ref: 50
Journal code: 8112917. ISSN: 0100-879X.
AB **G protein-coupled receptor** (GPCR) **activation** is followed rapidly by adaptive changes that serve to diminish the responsiveness of a cell to further stimulation. This process, termed desensitization, is the consequence of receptor phosphorylation, **arrestin** binding, sequestration and down-regulation. GPCR phosphorylation is initiated within seconds to minutes of receptor **activation** and is mediated by both second messenger-dependent protein kinases and receptor-specific **G protein-coupled receptor** kinases (GRKs). Desensitization in response to GRK-mediated phosphorylation involves the binding of **arrestin** proteins that serve to sterically uncouple the receptor from its G protein. GPCR sequestration, the endocytosis of receptors to endosomes, not only contributes to the temporal desensitization of GPCRs, but plays a critical role in GPCR resensitization. GPCR down-regulation, a loss of the total cellular complement of receptors, is the consequence of both increased lysosomal degradation and decreased mRNA synthesis of GPCRs. While each of these agonist-mediated desensitization processes are initiated within a temporally dissociable time frame, recent data suggest that they are intimately related to one another. The use of **green fluorescent protein** from the jellyfish Aqueora victoria as an epitope tag with intrinsic fluorescence has facilitated our understanding of the relative relationship between GRK phosphorylation, **arrestin** binding, receptor sequestration and down-regulation.

L11 ANSWER 4 OF 12 MEDLINE on STN
TI A beta-**arrestin/green fluorescent**

protein biosensor for detecting **G protein-coupled receptor activation.**

PY 1997

AU Barak L S; Ferguson S S; Zhang J; Caron M G

SO Journal of biological chemistry, **(1997 Oct 31)** 272 (44) 27497-500.
Journal code: 2985121R. ISSN: 0021-9258.

AB **G protein-coupled receptors** (GPCR) represent the single most important drug targets for medical therapy, and information from genome sequencing and genomic data bases has substantially accelerated their discovery. The lack of a systematic approach either to identify the function of a new GPCR or to associate it with a cognate ligand has added to the growing number of orphan receptors. In this work we provide a novel approach to this problem using a beta-arrestin2/**green fluorescent protein** conjugate (betaarr2-GFP). It provides a real-time and single cell based assay to monitor GPCR **activation** and GPCR-**G protein-coupled receptor** kinase or GPCR-**arrestin** interactions. Confocal microscopy demonstrates the translocation of betaarr2-GFP to more than 15 different ligand-activated GPCRs. These data clearly support the common hypothesis that the beta-**arrestin** binding of an activated receptor is a convergent step of GPCR signaling, increase by 5-fold the number of GPCRs known to interact with beta-arrestins, demonstrate that the cytosol is the predominant reservoir of biologically active beta-arrestins, and provide the first direct demonstration of the critical importance of **G protein-coupled receptor** kinase phosphorylation to the biological regulation of beta-**arrestin** activity and GPCR signal transduction in living cells. The use of betaarr2-GFP as a biosensor to recognize the **activation** of pharmacologically distinct GPCRs should accelerate the identification of orphan receptors and permit the optical study of their signal transduction biology intractable to ordinary biochemical methods.

L11 ANSWER 5 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

TI **Activation** and desensitisation of the thyrotropin-releasing hormone receptor visualised by monitoring cellular redistribution of a beta-**arrestin**-1-**green fluorescent protein** fusion protein.

PY **1999**

AU Groarke, Alex [Reprint author]; Milligan, Graeme [Reprint author]

SO Biochemical Society Transactions, (1999) Vol. 27, No. 3, pp. A118. print.
Meeting Info.: 668th Meeting of the Biochemical Society. Glasgow, Scotland, UK. April 7-9, 1999. Biochemical Society.
CODEN: BCSTB5. ISSN: 0300-5127.

L11 ANSWER 6 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

TI Real-time visualization of the cellular redistribution of **G protein-coupled receptor** kinase 2 and beta-**arrestin** 2 during homologous desensitization of the substance P receptor.

PY **1999**

AU Barak, Larry S.; Warabi, Kengo; Feng, Xiao; Caron, Marc G.; Kwatra, Madan M. [Reprint author]

SO Journal of Biological Chemistry, (March 12, 1999) Vol. 274, No. 11, pp. 7565-7569. print.
CODEN: JBCHA3. ISSN: 0021-9258.

AB The substance P receptor (SPR) is a **G protein-coupled receptor** (GPCR) that plays a key role in pain regulation. The SPR desensitizes in the continued presence of agonist, presumably via mechanisms that implicate **G protein-coupled receptor** kinases (GRKs) and beta-arrestins. The temporal relationship of these proposed biochemical events has never been established for any GPCR other than rhodopsin beyond the resolution

provided by biochemical assays. We investigate the real-time **activation** and desensitization of the human SPR in live HEK293 cells using **green fluorescent protein** conjugates of protein kinase C, GRK2, and beta-**arrestin** 2. The translocation of protein kinase C betaII-**green fluorescent protein** to and from the plasma membrane in response to substance P indicates that the human SPR becomes activated within seconds of agonist exposure, and the response desensitizes within 30 s. This desensitization process coincides with a redistribution of GRK2 from the cytosol to the plasma membrane, followed by a robust redistribution of beta-**arrestin** 2 and a profound change in cell morphology that occurs after 1 min of SPR stimulation. These data establish a role for GRKs and beta-arrestins in homologous desensitization of the SPR and provide the first visual and temporal resolution of the sequence of events underlying homologous desensitization of a GPCR in living cells.

L11 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Using **green fluorescent protein** to understand the mechanisms of **G-protein-coupled receptor** regulation.
PY **1998**
AU Ferguson, S. S. G. [Reprint author]
SO Brazilian Journal of Medical and Biological Research, (Nov., 1998) Vol. 31, No. 11, pp. 1471-1477. print.
CODEN: BJMRDK. ISSN: 0100-879X.
AB **G protein-coupled receptor** (GPCR) **activation** is followed rapidly by adaptive changes that serve to diminish the responsiveness of a cell to further stimulation. This process, termed desensitization, is the consequence of receptor phosphorylation, **arrestin** binding, sequestration and down-regulation. GPCR phosphorylation is initiated within seconds to minutes of receptor **activation** and is mediated by both second messenger-dependent protein kinases and receptor-specific **G protein-coupled receptor** kinases (GRKs). Desensitization in response to GRK-mediated phosphorylation involves the binding of **arrestin** proteins that serve to sterically uncouple the receptor from its G protein. GPCR sequestration, the endocytosis of receptors to endosomes, not only contributes to the temporal desensitization of GPCRs, but plays a critical role in GPCR resensitization. GPCR down-regulation, a loss of the total cellular complement of receptors, is the consequence of both increased lysosomal degradation and decreased mRNA synthesis of GPCRs. While each of these agonist-mediated desensitization processes are initiated within a temporally dissociable time frame, recent data suggest that they are intimately related to one another. The use of **green fluorescent protein** from the jellyfish Aqueora victoria as an epitope tag with intrinsic fluorescence has facilitated our understanding of the relative relationship between GRK phosphorylation, **arrestin** binding, receptor sequestration and down-regulation.

L11 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI A beta-**arrestin/green fluorescent protein** biosensor for detecting **G protein-coupled receptor activation.**
PY **1997**
AU Barak, Larry S.; Ferguson, Stephen S. G.; Zhang, Jie; Caron, Marc G. [Reprint author]
SO Journal of Biological Chemistry, (Oct. 31, 1997) Vol. 272, No. 44, pp. 27497-27500. print.
CODEN: JBCHA3. ISSN: 0021-9258.
AB **G protein-coupled receptors** (GPCR) represent the single most important drug targets for medical therapy, and information from genome sequencing and genomic data bases has

substantially accelerated their discovery. The lack of a systematic approach either to identify the function of a new GPCR or to associate it with a cognate ligand has added to the growing number of orphan receptors. In this work we provide a novel approach to this problem using a beta-arrestin2/**green fluorescent protein** conjugate (betaarr2-GFP). It provides a real-time and single cell based assay to monitor GPCR **activation** and GPCR-**G protein-coupled receptor** kinase or GPCR-**arrestin** interactions. Confocal microscopy demonstrates the translocation of betaarr2-GFP to more than 15 different ligand-activated GPCRs. These data clearly support the common hypothesis that the beta-**arrestin** binding of an activated receptor is a convergent step of GPCR signaling, increase by 5-fold the number of GPCRs known to interact with beta-arrestins, demonstrate that the cytosol is the predominant reservoir of biologically active beta-arrestins, and provide the first direct demonstration of the critical importance of **G protein-coupled receptor** kinase phosphorylation to the biological regulation of beta-**arrestin** activity and GPCR signal transduction in living cells. The use of betaarr2-GFP as a biosensor to recognize the **activation** of pharmacologically distinct GPCRs should accelerate the identification of orphan receptors and permit the optical study of their signal transduction biology intractable to ordinary biochemical methods.

L11 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
TI Real-time visualization of the cellular redistribution of **G protein-coupled receptor** kinase 2 and β-**arrestin** 2 during homologous desensitization of the substance P receptor
PY **1999**
AU Barak, Larry S.; Warabi, Kengo; Feng, Xiao; Caron, Marc G.; Kwatra, Madan M.
SO Journal of Biological Chemistry (**1999**), 274(11), 7565-7569
CODEN: JBCHA3; ISSN: 0021-9258
AB The substance P receptor (SPR) is a **G protein-coupled receptor** (GPCR) that plays a key role in pain regulation. The SPR desensitizes in the continued presence of agonist, presumably via mechanisms that implicate **G protein-coupled receptor** kinases (GRKs) and β-arrestins. The temporal relationship of these proposed biochem. events has never been established for any GPCR other than rhodopsin beyond the resolution provided by biochem. assays. The authors investigate the real-time **activation** and desensitization of the human SPR in live HEK293 cells using **green fluorescent protein** conjugates of protein kinase C, GRK2, and β- **arrestin** 2. The translocation of protein kinase C βII- **green fluorescent protein** to and from the plasma membrane in response to substance P indicates that the human SPR becomes activated within seconds of agonist exposure, and the response desensitizes within 30 s. This desensitization process coincides with a redistribution of GRK2 from the cytosol to the plasma membrane, followed by a robust redistribution of β- **arrestin** 2 and a profound change in cell morphol. that occurs after 1 min of SPR stimulation. These data establish a role for GRKs and β-arrestins in homologous desensitization of the SPR and provide the first visual and temporal resolution of the sequence of events underlying homologous desensitization of a GPCR in living cells.

L11 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
TI Using **green fluorescent protein** to understand the mechanisms of **G-protein-coupled receptor** regulation
PY **1998**
AU Ferguson, S. S. G.

SO Brazilian Journal of Medical and Biological Research (**1998**), 31(11), 1471-1477
CODEN: BJMRDK; ISSN: 0100-879X

AB A review, with 50 refs. **G protein-coupled receptor** (GPCR) **activation** is followed rapidly by adaptive changes that serve to diminish the responsiveness of a cell to further stimulation. This process, termed desensitization, is the consequence of receptor phosphorylation, **arrestin** binding, sequestration and down-regulation. GPCR phosphorylation is initiated within seconds to minutes of receptor **activation** and is mediated by both second messenger-dependent protein kinases and receptor-specific **G protein-coupled receptor** kinases (GRKs). Densensitization in response to GRK-mediated phosphorylation involves the binding of **arrestin** proteins that serve to sterically uncouple the receptor from its G protein. GPCR sequestration, the endocytosis of receptors to endosomes, not only contributes to the temporal desensitization of GPCRs, but plays a critical role in GPCR resensitization. GPCR down-regulation, a loss of the total cellular complement of receptors, is the consequence of both increased lysosomal degradation and decreased mRNA synthesis of GPCRs. While each of these agonist-mediated desensitization processes are initiated within a temporally dissociable time frame, recent data suggest that they are intimately related to one another. The use of **green fluorescent protein** from the jellyfish Aqueora victoria as an epitope tag with intrinsic fluorescence has facilitated our understanding of the relative relationship between GRK phosphorylation, **arrestin** binding, receptor sequestration and down-regulation.

L11 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

TI Assaying receptor activity using constructs expressing β-**arrestin** conjugates with **green fluorescent protein**

PY 1998
2003
1999
1998
1998
2003
2000
2004
2004
2004
2004

IN Barak, Lawrence S.; Caron, Marc G.; Ferguson, Stephen S.; Zhang, Jie

SO PCT Int. Appl., 60 pp.
CODEN: PIXXD2

AB Described are methods of detecting **G-protein coupled receptor** (GPCR) activity in vivo and in vitro; methods of assaying GPCR activity; and methods of screening for GPCR ligands, **G protein-coupled receptor** kinase (GRK) activity, and compds. that interact with components of the GPCR regulatory process. The assays are based on the observation that β- **arrestin** redistribution from the cytosol to the plasma membrane occurs in response to agonist **activation** of GPCRs, demonstrating a common role for β- **arrestin** in agonist-mediated signal transduction termination. Thus, translocation of conjugates of a β- **arrestin** protein with a detectable mol. indicates **activation** of the GPCR pathway. A fusion protein consisting of a β- **arrestin** mol. (β-arrestin2) conjugated to a **green fluorescent protein** (S65T variant) at its C-terminus is expressed in cells and is biol. active. This conjugates allows convenient methods of assaying agonist stimulation of GPCRS in vivo and in vitro in real time.

L11 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

TI A β- **arrestin/green fluorescent protein** biosensor for detecting **G protein-coupled receptor activation**

PY **1997**

AU Barak, Larry S.; Ferguson, Stephen S. G.; Zhang, Jie; Caron, Marc G.

SO Journal of Biological Chemistry (**1997**), 272(44), 27497-27500
CODEN: JBCHA3; ISSN: 0021-9258

AB **G protein-coupled receptors** (GPCR) represent the single most important drug targets for medical therapy, and information from genome sequencing and genomic data bases has substantially accelerated their discovery. The lack of a systematic approach either to identify the function of a new GPCR or to associate it with a cognate ligand has added to the growing number of orphan receptors. In this work we provide a novel approach to this problem using a β-arrestin2/ **green fluorescent protein** conjugate (βarr2-GFP). It provides a real-time and single cell based assay to monitor GPCR **activation** and GPCR-**G protein-coupled receptor** kinase or GPCR-**arrestin** interactions. Confocal microscopy demonstrates the translocation of βarr2-GFP to more than 15 different ligand-activated GPCRs. These data clearly support the common hypothesis that the β-**arrestin** binding of an activated receptor is a convergent step of GPCR signaling, increase by 5-fold the number of GPCRs known to interact with β-arrestins, demonstrate that the cytosol is the predominant reservoir of biol. active β-arrestins, and provide the first direct demonstration of the critical importance of **G protein-coupled receptor** kinase phosphorylation to the biol. regulation of β- **arrestin** activity and GPCR signal transduction in living cells. The use of βarr2-GFP as a biosensor to recognize the **activation** of pharmacol. distinct GPCRs should accelerate the identification of orphan receptors and permit the optical study of their signal transduction biol. intractable to ordinary biochem. methods.